ACERIA TULIPAE (KEIFER) (ERIOPHYIDAE) IN RELATION TO THE TRANSMISSION OF VARIOUS STRAINS OF WHEAT STREAK MOSAIC VIRUS

by

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INTRODUCTION

Wheat streak mosaic is a very serious virus disease of wheat in the Great Plains. It was observed as early as 1929 in Kansas and probably before that in Nebraska (McKinney, 1932). The disease was reported in 1930 in northwestern Kansas and caused losses in many acres in Rawlins and Sheridan Counties. About 800 acres of wheat in 1943 and 5000 in 1948 were severely attacked by the disease in western Kansas. In Ness County, 75 per cent of the crop was virtually destroyed in 1948 (Hansing, et al., 1950). In 1949, the disease caused heavy losses in several areas in the state, amounting to approximately 30 million dollars (Haskett, et al., 1956). These losses stimulated research in Kansas and several other western states and from 1949 to 1953 an intensive search for possible vectors was conducted.

The transmission of the virus by an aphid, Toxoptera graminum (Rond.) was reported by Atkinson (1949) but could not be confirmed by others. At Kansas State College Painter and Fellows, during the fall and winter of 1949, using the same aphid, and five other species, obtained no transmission in any experiment (unpublished report). Harvey (1951) tested various insects as possible vectors but failed to obtain consistent positive results. He suggested the possibility of a certain leafhopper as the vector but was not able to prove this conclusively.

Slykhuis (1953) discovered that the eriophyid mite, Aceria tulipae (Keifer), transmitted wheat streak mosaic virus. In another series of experiments (1955) he confirmed the ability of A. tulipae (K.), to carry the disease. Connin in Kansas and Staples in Nebraska (unpublished report) have also recently confirmed the report of Slykhuis. This discovery was

of great value and led to the realization of the economic importance of this mite in wheat production.

McKinney (1944) indicated the presence of probable strains of wheat streak mosaic virus. Recent unpublished findings at Kansas State College have shown that there are possibly at least five strains of the virus. Whether these strains of wheat streak mosaic virus could be transmitted by A. tulipae (K.), was not known. It was important to know whether each could be transmitted with equal efficiency. This investigation was undertaken primarily to answer these two questions and secondarily to add wherever possible to our knowledge concerning the life cycle and habits of the mite.

REVIEW OF LITERATURE

Amos, Halton, Knight and Massee in 1927 (Leach, 1940, pp. 369-371), presented evidence for the transmission of the currant reversion virus by the currant bud mite. This report was probably the first account of eriophyid mites as vectors of plant virus diseases. However, the results were questioned as working with eriophyid mites offered certain difficulties. The recognition of eriophyid mites as vectors of plant viruses did not gain much foothold until Slykhuis (1953) demonstrated the efficiency of Aceria tulipae (K.), as the vector of wheat streak mosaic virus.

The failure by plant pathologists and entomologists prior to 1953, to recognize the role of eriophyid mites as vectors is probably because of their size. They are very small, about 270 microns in length, and are hardly visible to the unaided eye when occurring singly. They must be magnified 20 to 50 times to be studied. Hassan (1928) and Keifer (1938) described the family. They are worm-like with a cylindrical, elongate body.

They differ from the other Acarinas in that they have only two pairs of legs instead of four. These are located at the anterior end of the body. Baker (1952) discussed the eriophyid mites in general and A. tulipae (K.) in particular as follows:

> Eriophyids exhibit a very intimate mite-host relationship, characterized by considerable host specificity. Gall formation of one kind or another is another aspect of this intimacy, but the majority depend on natural formations on their hosts and cause no discernable injury.
>
> The eriophyid with the most peculiar habitat that has come to notice so far, is the onion or bulb eriophyid, Aceria tulipae (Keifer). Liliaceous bulbs such as onions, garlics and tulips are attacked, the mites living between the bulb layers. Thus the mite lives underground, a habit not known to be possessed by any other species. The activities of A. tulipae cause the bulb to shrink and dry out. These mites persist in storage.

Since the discovery of Slykhuis, fig mosaic in California has been reported to be transmitted by Aceria ficus (Keifer) (Flock and Wallace, 1955) and an eriophyid mite of the genus Eriophyes, apparently an undescribed species, has also been found to be a vector of the peach-mosaic virus (Wilson, et al., 1955).

Kido and Stafford (1955) reported the association of eriophyid mites in grape buds. They observed that the mites were confined mostly to the periphery of the bud scales and to the crevices formed by the folds on the bud scales.

A. tulipae (K.) was first collected from tulip bulbs at Sacramento, California and was named by Keifer (1938). The mite is believed to have come from Holland in tulip bulb shipments. In stored bulbs, the mites were found to be concentrated particularly on the green growing tips and to some extent the white bracts (Lange, 1955).

Slykhuis (1953) first reported A. tulipae (K.) on wheat plants. Since that report it has been found on wheat by many workers throughout the Great

Plains. In wheat plants, Kantack and Knutson (1954) found the mites congregating on the bases of the leaf sheath including the pocket formed by the ligule and they were also present on green seeds and various leaf areas.

Batchelor (1952) thought that eriophyid mites were dependent upon perennial hosts for their survival which may be true of A. tulipae (K.), since an over-summering perennial host, western wheat grass, Agropyron smithii (Rydb.) has been reported (Painter and Schesser, 1954). Mite colonies were abundant in the whorls of non-fruiting stems of this grass and also along the deep interveinal crevices.

Grasses commonly found naturally infected with wheat streak mosaic virus in the field in Nebraska, were infested with mites in the greenhouse, but proved to be poor hosts of A. tulipae (K.) (Staples and Allington, 1956). Mites were rarely observed staying on wild grasses tested more than four days after infestation, and eggs were not laid on them (Connin, 1956, a). A. tulipae (K.) found in nature on Hordeum jubstum (L.), Agropyron smithii (Rydb.), and Elymus canadensis (L.), failed to increase in number when transferred to wheat plants. Similarly, attempts to rear mites from wheat on those grasses were unsuccessful (Slykhuis, 1955). Reports to date would indicate that most grasses with the exception of possibly A. smithii are temporary hosts of the mites probably until they can find a suitable host like volunteer wheat (Connin, 1956, b).

The movement of A. tulipae (K.) in the air was shown by Pady (1955) and Slykhuis (1955). Pady collected mites on smeared slides exposed to the air on top of a three story building. The nearest wheat fields to the south were 1½ miles away. Slykhuis placed vaseline-coated microscope slides in the path of air blowing (as supplied by a 10-inch electric fan) past mite-infested wheat in the greenhouse and found mites on the slides. Connin (1956, b) in

a periodic examination of wheat fields obtained evidence that the mites were carried into the wheat fields by the wind from nearby volunteer wheat.

The life cycle of the mite was reported by Slykhuis (1955) to be seven days. Staples and Allington (1956) estimated the complete life cycle from egg to egg to be as follows:

Egg incubation period	3 days
First nymph	1½ days
First molt	¾ day
Second nymph	1½ days
Second molt	¾ day
Egg hatching to adult	4 to 5 days
Preoviposition period	1 to 2 days
Complete cycle from egg to egg	8 to 10 days

Wheat streak mosaic virus was readily transmitted on wheat by A. tulipae (K.), in all stages except through the eggs (Slykhuis, 1955). Apparently the mite often carried an additional chlorotic leaf factor, possibly a virus, which was not transmitted manually (Slykhuis, 1955). Other eriophyid mites have also produced similar symptoms. Wilson and Cochran (1952) reported that an eriophyid mite was responsible for the well-known silvering of peach foliage. Before the discovery of the transmission of fig mosaic by a mite, the mottling and distortion of leaves were associated with the mite (Flock and Wallace, 1955). Wheat plants infested with wheat curl mites are usually characterized by longitudinally rolled leaves, the tips of which become trapped in the rolled leaves and sheaths. Slykhuis (1955) reported that this characteristic symptom may be present on healthy wheat plants infested with nonviruliferous mites.

MATERIALS AND METHODS

Virus Strains

Five different probable strains of wheat streak mosaic virus under code

numbers MS, UK, SC, SD-1 and 41 were obtained from the stock cultures at Kansas State College as sources of inoculum. The inocula in these experiments were prepared by extracting the juice from the leaves of Pawnee wheat plants infected with these separate strains. The infected leaves were macerated in a mortar and pestle with quartz sand and diluted with water mixed with carborundum dust. For every gram of infected leaves, 10 ml. of water was diluted making the dilution roughly 1 to 10.

Mites Used

The mites used in these investigations were secured from wheat plants in the fields. Since it was necessary to use nonviruliferous mites for these experiments, only newly laid eggs were picked up from field cultures with medium coarse human hair and placed on healthy wheat plants. The newly-hatched nymphs from these eggs were then transferred to other healthy and and diseased wheat plants as needed. Mites were sent to H. H. Keifer, in California for species identification.[1/]

Plants Used

Pawnee wheat planted in 4- and 6- inch pots was the only variety used. The seeds were carefully covered with Spergon dust prior to placing them in sterilized Petri dishes lined with moistened filter paper. After the seeds germinated, the seedlings were planted in potted steam-sterilized soil. Biweekly plantings were made so that enough plants would be available for the experiments. Virus infected plants were obtained by inoculating young

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1/Systematic entomologist of the California State Department of Agriculture, Sacramento 14, California.

healthy seedlings with the extracted juice obtained from the mosaic infected plants mentioned previously.

Greenhouse Facilities

The mite transmission work was done in the northeast section of the mosaic greenhouse at Kansas State College. During the course of the investigation, all plants were placed in the various cages in this greenhouse. None was grown in the open. Other sections where there were no mite experiments in progress were used as starting rooms for the healthy plants and for maintaining the cultures of manually infected wheat streak mosaic plants. The starting room was fumigated with Plantfume 103 (a smoke generator, active ingredient tetraethyl dithiopyrophosphate, 15 per cent) every week to protect the plants as much as possible from insects and mites and was kept entirely closed during the winter. Only young plants were allowed in this room. This along with the fumigations prevented the development of insect and mite populations. The daily temperature of the greenhouse varied greatly but averaged about 70°F. in the winter when the automatic heating system could function. During the summer months, the temperatures in the daytime were often more than 100°F., even when the greenhouses were covered heavily with whitewash. The cement floor and wooden benches were constantly moistened to maintain high humidity and to cool the rooms somewhat. Some insects and mites came through the open upper ventilators during the summer but consistent use of control measures available held populations down. The side ventilators were screened with 36-mesh lumite screen which could exclude larger insects such as aphids but not the mites.

Transmission Cages Used

There were five different types of cages used in these experiments. The first one was built by R. V. Connin who obtained the idea from the University of Nebraska, Department of Plant Pathology. These were made of a wooden frame with dimensions of 18 x 24 inches and were covered on three sides with nylon taffeta (Fig. 1 of Plate I). A plastic sheet having three orifices closed by rubber stoppers served as a front. These cages were fitted tightly to 6-inch clay flower pots by means of a 6-inch diameter groove which was filled with Armstrong caulking compound.

The second type of cage consisted of lamp-globes covered on top with nylon taffeta (Fig. 2 of Plate I). The nylon taffeta was pasted on top with caulking compound and held in place by two rubber bands. The base of the lamp chimney was then pushed into the soil around the growing plants.

The third cages were developed by Fellows and Connin (1952) and were ground edged pyrex glass cylinders 8 inches in diameter and 12 inches long with plastic tops set in Armstrong's caulking compound (Plate II). These cages were arranged in a row and cemented to a bench. Underneath the bench, a series of rubber manifolds, 1½ inches in diameter, were provided and attached to a master manifold of galvanized iron, connected to a Dayton blower with a capacity of 150 cubic feet of air per minute. Each cage had an individual air inlet and outlet, each covered with 36-mesh lumite screen. The air inlet which was attached to the rubber manifold had a hand operated valve to control the flow of the air current. The pots in the cages were watered from below by means of brass and rubber tubings which were opened and closed by pinch-cocks. These rubber tubings were in turn connected to a reservoir of water set high enough so that a gravity flow system to each

EXPLANATION OF PLATE I

Fig. 1. Transmission cage made of wooden frame and covered on three sides with nylon taffeta. A plastic sheet having three orifices closed by rubber stoppers served as a front.

Fig. 2. Lamp-globe covered with nylon taffeta on the top served as another cage. The nylon taffeta was pasted on the top with Armstrong's caulking compound and held in place with a rubber band. The base of the lamp chimney was pushed into the soil.

PLATE I

Fig. 1 (back row)

Fig. 2 (front row)

EXPLANATION OF PLATE II

Another type of transmission cage is ground edge pyrex glass cylinders 8 inches in diameter and 12 inches long with plastic tops set in Armstrong's caulking compound. The cage is cemented to a bench. Underneath the bench are a series of rubber manifolds which are attached to a master manifold of galvanized iron and connected to a Dayton blower. The pots in the cages are watered from below by means of brass and rubber tubings which are opened and closed by pinch-cocks. These rubber tubings are in turn connected to a reservoir of water.

PLATE II

pot operated when the pinch-cocks were opened.

Large test tubes, 2 cm. in diameter and 8 inches long plugged with rubber sponge stoppers and inverted in Hyponex plant nutrient solution (Hydroponic Chemical Inc., 75 grams to a gallon of water), were used as starting cages (Plate III).

Large wooden framed glass cages measuring 33 x 31 x 23 inches were also used (Plate IV). These were developed by Fellows and Connin (1952). They had a wood bottom and one wood end. Two orifices, two inches in diameter, were located in the wood end near the top; one was the air outlet and the other was used for watering the plants inside the cages. The air outlet was covered with a 36-mesh lumite screen and the watering opening was plugged with a 2-inch rubber stopper. Air was supplied from a master blower, described previously, through a tube of galvanized iron, 27 inches long with a brass casting. The end of the galvanized iron tube inside the cage had 12 air inlet apertures. The cages opened at the top by means of an 11 x 31 inch wooden access door hinged to the frame.

Miscellaneous Equipment Used

The pots in these experiments were sterilized in a low pressure steam sterilizer for two hours and then wrapped in clean brown wrapping paper. All soil used was sterilized in the autoclave for two hours under 15 pounds pressure. Since the mites were hardly visible to the naked eye, a binocular microscope magnifying from 20 to 40 times was used for all mite studies and transfers. Strands of medium coarse human hair were used in picking up and moving the mites.

All equipment used was carefully sterilized in a steam sterilizer. All cages were washed in clorox water (three teaspoons to a quart of water) and

EXPLANATION OF PLATE III

Large test tubes, 2 cm. in diameter and 8 inches long plugged with rubber sponge stoppers inverted in a wire basket and placed over a clay saucer with Hyponex plant nutrient solution, are used as starting cages.

PLATE III

EXPLANATION OF PLATE IV

A large wooden framed glass cage used in the transmission studies. The long galvanized iron tube inside the cage has 12 air inlet apertures. The cage opens at the top by means of an 11 x 31 inches wooden access door hinged to the frame.

PLATE IV

then wiped with a rubber sponge which had been dipped in 95 per cent ethyl alcohol. The hands were washed with luke warm water and soap and then disinfected with 95 per cent ethyl alcohol before and between each separate experiment. All plants infested with mites were protected from strong air currents by the cages. No uncaged mite infested plants were allowed in the greenhouse section used.

Collecting and Rearing of Mites

Mites found in wheat fields were brought to the greenhouse. The infested plants were examined under the binocular microscope for eggs. These eggs found were placed on healthy excised wheat leaves in sterilized Petri dishes lined with moistened filter paper. The leaves were then examined twice a day for hatched eggs. The newly-hatched nymphs were then transferred to 2-week-old wheat plants growing in the inverted test tube cage already described. The location on the leaves where the mites were placed was marked with India ink to facilitate future examinations under the binocular microscope. After 48 hours in the inverted test tube cages, the plants upon which mites were thriving were transplanted to the lamp-globe cages. These plants were then examined under the binocular microscope twice a week and were grown in these cages until good colonies of mites were established. Then the plants were transferred finally to the nylon taffeta or cylindrical glass cages and the mite cultures were maintained in them.

Comparison of Cotton and Rubber Sponges as Plugs in the Inverted Test Tube Cages

Absorbent cotton was cut into strips small enough to fit the mouth of the 2 cm. test tubes. Rubber sponges, 3 x 5 inches were cut into small

squares which fitted the same test tubes. A slit was made in the center where the seedlings could be inserted. These two types of plugs were then compared during the early experimental period to see whether the mite development was affected in any way.

Method of Transferring the Mites

Since A. tulipae (K.), is a very small mite, it was necessary to find a good method of transferring the mites from one place to another. The following methods were investigated:

A. Transfer by Light. When leaves covered with mites were placed over the leaves of uninfested plants and light from a 50 watt incandescent light bulb held about 5 inches away was directed at them, the mites moved away from the light. In other experiments a flashlight was used instead of the incandescent light bulb. The mites were observed constantly under the binocular microscope during these experiments. The mites were apparently negatively phototropic and always moved away from the light as rapidly as possible. After a definite number had migrated to the uninfested leaves the light and the leaves to which the mites had migrated were removed.

B. Transfer by the Use of Medium Coarse Human Hair. Eggs were transferred easily by using hair with a hollow tip, while the adults and nymphs were removed with hair having pointed ends. Various types of hair were tested and the medium coarse types worked best. Slykhuis (1955) used squirrel hair of suitable flexibility glued to a wooden handle about the size of a pencil in transferring individual mites or eggs.

C. Transfer by Movement of Infested Leaf Portions. Wheat leaves were examined under the binocular microscope for mite colonies. When a sufficient

number was located, the leaf was cut with a pair of scissors and then the cut portion was inserted in the youngest leaf axil (preferably the unfolded one) of an uninfested wheat plant with a pair of forceps and clamped on with a woman's hair clip.

Transmission Experiments

To obtain cultures of viruliferous mites, non-viruliferous mites were transferred by method C to manually inoculated diseased plants infected with different strains of the wheat streak mosaic virus. The diseased plants with the mites were then caged in the large nylon or cylindrical glass cages. In another set of experiments, healthy plants infested with non-viruliferous mites were inoculated with the extracted juice of the different strains of the virus. The virus preparations of the different strains were inoculated by dipping the thumb and index fingers in the inoculum and rubbing lightly on the leaves (McKinney, 1949). The plants were then caged in nylon taffeta or cylindrical glass cages and observed for symptoms.

To check on the efficiency of the transfer of the virus by the mite, a number of mites (from 1 to 10) from the above mentioned infested and diseased plants were transferred to 2-week-old healthy plants growing in the test tube cages. Only mites that were moving were picked up with the hair so that the mouth parts would not be injured. Since most mites did not move actively, light was directed at them to encourage movement. The mites were always placed at the base of the leaf or on the youngest unfolded leaf. After two days in the test tube cages, the plants carrying the supposed viruliferous mites were transplanted to lamp-globe cages. Before these transfers were made, the plants were examined to see if the mites were still alive. Each day thereafter the plants were examined for symptoms. When mosaic symptoms

appeared, the plants were again transplanted into pots in the nylon taffeta or cylindrical glass cages, for further observations. When the symptoms were fully developed, the infected leaves were macerated, the juice extracted, and inoculations were made to healthy wheat plants by the rubbing method just described. These manually inoculated plants were placed in another section of the greenhouse and were observed daily for the development of mosaic symptoms.

Two sets of controls were maintained in each experiment. In one lot, comparable healthy plants were maintained in the absence of mites. On the other, non-viruliferous mites were introduced and maintained on comparable healthy plants. In each case the same treatment, except for infection, was given to the controls as to the experimental plants and they were of the same number, age and variety.

In order to eliminate the possibility of mixture of different strains of the virus, all the equipment used and the hands were carefully washed with soap and water and then disinfected with 95 per cent ethyl alcohol before and after each transfer. The controls were always handled before the other experimental plants.

Possible Transmission by Eggs

An effort was made to find out whether the virus was transmitted through the eggs. Eggs from viruliferous mites were used. A definite number of eggs, from 6 to 12, were placed on healthy wheat plants grown in inverted test tube cages. The eggs were examined daily under the binocular microscope. When all the eggs had hatched, the plants were transferred to the lamp-globe cages. After the plants showed trapped leaves, the mite infested plants were transferred to the large glass cages and were observed for at least a month. If

symptoms had not developed in that time on the plants, the mites were considered to be nonviruliferous.

Two sets of controls were provided; the first set of plants did not have any eggs placed on them at all and the other group of plants had nonviruliferous eggs placed on them. Otherwise the treatment was the same. The same numbers of eggs and plants in the experimental lot were also used in the controls.

EXPERIMENTAL RESULTS

Rearing of Mites

In the early period of the experiments numerous attempts to rear individual mites under greenhouse conditions using various techniques were unsuccessful. Although eggs that were placed on excised wheat leaves in sterilized Petri dishes hatched, the majority of the nymphal forms when transferred to the large nylon taffeta or cylindrical glass cages did not survive. In an attempt to find an ideal environment favorable for the survival of the mites, wheat plants were grown in the inverted test tubes, described previously, and infested with the newly-hatched nymphs. It was observed that the nymphs in those cages were able to live for more than two days. However, the humidity became very high and the moisture inside the tubes condensed so much that the plant leaves were constantly soaked, and the mites appeared to be dying because of the excessive water. In an attempt to correct this condition the tubes were changed twice a day. In an effort to maintain a comparable environment to that of the test tube cages during the first two days these plants were transplanted to the lamp-globe cages. When plants showed trapped leaves in the lamp-globe cages and were too big

for normal development in them, they were again transplanted to the nylon taffeta or glass cylinder cages. This method of rearing the mites was consistently successful.

Comparison of Cotton and Rubber Sponges as Plugs in the Inverted Test Tube Cages

It was found that cotton plugs permitted excessive flow of the water inside the test tube cages, thus the base of the ligule or axil of the youngest leaf where the mites were usually placed was always filled with water. When this condition persisted, the mites apparently drowned or at least, did not survive or increase. When the rubber sponge plugs, described previously, were used the flooding was minimized and mites not only survived but increased (Table 7 in Appendix).

Transferring Mites

To determine a good method of transferring mites, various methods were tested. It was observed that mites apparently disliked bright light, as they tended to move away from it. The mites could be moved readily by this method and were not injured during the transfer since they moved voluntarily. However, this method was slow and it was hard to count the number of mites transferred.

The use of medium coarse human hair was found to be very fine for transferring mites. Less time was consumed and an exact number of mites could be moved. In picking up the eggs, a hollow tip was found to be the most ideal. The eggs fitted snugly in the hollow, making the transfer most convenient.

The third method, that of inserting a portion of an infested leaf into the leaf axils of an uninfested wheat plant was not very effective. The

mites would not move actively from the excised host tissue to the new wheat plant so that often, the mites died in place when the transferred leaf portion of the host withered. Also, it was quite difficult to determine the exact time when mites migrated to the new wheat plants. This method of transfer was not used in the transmission experiments since it was difficult to approximate the number of mites transferred and the actual time feeding began. This method of transfer was used only in infesting manually inoculated diseased plants to obtain cultures of viruliferous mites.

Transmission Experiments

The two methods of obtaining viruliferous mites already described, were found to be equally efficient, thus no effort was made to compare the two critically. However, nonviruliferous mites introduced to already manually inoculated diseased plants could be used for virus transmission 24 to 48 hours after introduction, while in the other method, it was necessary to wait until symptoms appeared on the infested plants before the mites could be used for transmission, and this usually took about 5 to 10 days.

A. tulipae (K.) was found to be an efficient vector of the five strains of wheat streak mosaic virus tested as shown in Tables 1 to 5 (in Appendix). From 1 to 10 mites were used in each test and a single mite readily transmitted the virus. The percentage of successful transmissions of the different strains varied from 84.21 to 92 per cent. The average incubation ranged from 7.04 to 8.68 days in the five strains. The maximum incubation period was 15 days and the minimum was 4 days. The symptoms produced by the various strains of the virus when transmitted by the mite were typical of those produced in manually inoculated plants under greenhouse conditions. A description

of these symptoms on Pawnee wheat essentially as given by Kainski (1955) follows.

SD-1. Faint green streaks and dashes on young leaves turn to white long bands and streaks on older leaves. On the older leaves the streaks and dashes become prominent and numerous. Some coalesce and form chlorotic areas and stripes white-yellow in color and irregular in shape. Infected leaves become typically elongated when compared with other collections. There is no marked stunting and proliferation or rolling of the leaves under greenhouse conditions (Plate V).

UK. Infected leaves are remarkable narrow and are covered with faint white-greenish streaks and dashes, which in older plants become white and yellow-white. There is a pronounced stunting effect, excessive proliferation and longitudinal rolling of infected leaves (Plate VI).

Al. This virus stunts the plants and stimulates proliferation of leaves, which remain short and broad. Blades of the infected leaves seldom roll or wrinkle except at very high temperatures. Light-green and yellow-green streaks and bands run along the entire leaf length. Mosaic mottling and crinkling of the leaves may be observed two to three weeks after inoculation. They later tend to disappear (Plate VII).

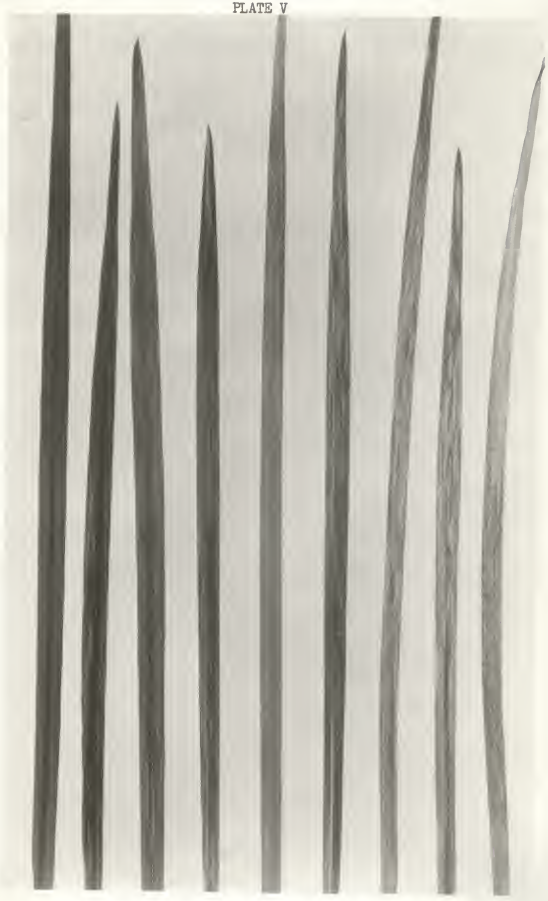
SC. The symptoms are very similar to those produced by UK. Leaves are usually rolled longitudinally and have streaks which are broadened and form white necrotic or yellowish spots. Stunting is evident and severe. Some plants may die. Proliferation is marked but not quite so prominent as in plants infected with UK virus (Plate VIII).

MS. Four to five days after inoculation distortion of the leaves commences, after which crinkling and mottling symptoms develop. The first leaves which emerge after inoculation are rolled with desiccated tips. The tips

EXPLANATION OF PLATE V

Symptoms produced by SD-1 strain of wheat streak mosaic virus, showing faint green streaks and dashes on young leaves and white long bands and streaks on older leaves.

PLATE V



EXPLANATION OF PLATE VI

UK Strain. Infected leaves are remarkably narrow and covered with faint white-greenish streaks and dashes. There is an excessive proliferation and longitudinal rolling of the leaves.

PLATE VI

EXPLANATION OF PLATE VII

Al strain. Light-green and yellow-green streaks and bands run along the entire leaf length.

PLATE VII

EXPLANATION OF PLATE VIII

SC strain. The leaves are usually longitudinally rolled. Streaks are broad and form white necrotic or yellowish spots.

PLATE VIII

usually wither, leaves developing later are distinctly shorter. Plants inoculated when young often die and when inoculated at the five to six leaf stage diseased leaves are shorter, erect and tend to be broader than normal. Small spots, dashes and streaks of yellow color develop on the infected leaves. These gradually increase in size and number. On older leaves they coalesce and form distinct chlorotic to yellow spots. The spots increase in size and sometimes all leaves become wholly yellow and then may die. These symptoms were typical only of the desiccated source of the MS virus. The greenhouse source was similar to 41 only more severe (Plate IX).

The fact that the mite produces an additional chlorotic factor as reported by Slykhuis (1955) was confirmed by the writer. However, this chlorotic factor can be recognized easily. It was localized in the areas where trapped leaves were found. The chlorotic region was yellowish becoming straw colored as the leaves unfolded. Also it was nontransmissable mechanically and was present on the healthy plants of the nonviruliferous mite cultures.

In no instance did the controls, healthy plants with nonviruliferous mites and healthy plants without any mites, develop any symptoms typical of wheat streak mosaic.

Transmission of the Virus by Eggs

By a series of experiments described previously it was possible to prove that the virus was not carried through the eggs of viruliferous mites (Table 6 in Appendix). A total of 142 eggs was used in these experiments. This confirms the report of Slykhuis (1955) that the eggs from viruliferous mites do not carry the virus.

EXPLANATION OF PLATE IX

MS strain. Crinkling and mottling develop on infected leaves. Small spots, dashes and streaks of yellow color are characteristic symptoms produced by this strain.

PLATE IX

DISCUSSION AND SUMMARY

The rearing of mites under greenhouse conditions presented a severe problem at first, most attempts being unsuccessful. This problem was solved by keeping the mites in a more humid environment. High humidity was maintained by using test tube and lamp-globe cages, described and illustrated in the text, as early colonizing chambers. When the mites were reproducing well and forming colonies, they were then transferred to large nylon taffeta or glass cylinder cages. The nylon taffeta cages were moistened often with water to maintain high humidity, especially during the summer. High humidity may be a favorable factor in the development of large mite populations in the field although information concerning the effect of humidity and temperature on the increase of large mite populations is still sparse. If these factors do affect the life cycle of the mite in the field, such information would be valuable to help predict rapid field increase in virus-vector populations.

Early in the experiment, cotton was used as plugs for the inverted test tube cages. It was observed that an excessive flow of water entered these cages thus soaking the leaves, particularly the ligules and leaf axils. Mite survival under these flooding conditions was very low (Table 7 in Appendix). With the use of rubber sponges as plugs, this flow of water was minimized and mites were able to survive. The evidence indicated that prolonged flooding was detrimental to the mites. The prolonged flooding of mite infested wheat fields might reduce populations drastically but would rarely occur in Kansas. Gibson (unpublished thesis, 1957), however, reported that dislodged mites could survive for at least 30 minutes in water. This would suggest that the mites to be killed must be submerged in water for a longer

period of time, as in the case of the inverted test tube cages having cotton plugs where the mites were under water for two days or more. It should also be remembered that a complete nutrient solution was dissolved in the water used with the inverted test tube cages. This might have been harmful to the mites, but the possibility was not checked.

Various methods of transferring individual mites were tested but failed. Finally, medium coarse human hair was found to be most useful in handling both individual eggs and adults.

Aceria tulipae (K.), proved to be negatively phototropic, the mites tending to move away when incandescent light or light from a flashlight was directed at them at close range. The fact that mites were found most abundantly on ligules and leaf axils of the plant (Kantack and Knutson, 1954) and were often lodged within the leafsheaths suggested that A. tulipae (K.), might aggregate in the darker places on the wheat plants, avoiding exposure to intense light. The fact that mites have been found on many occasions by various workers including the writer between the leaf bracts of onion bulbs and also among the crown roots of wheat plants would also support this contention.

Nonviruliferous mites placed on diseased plants infected with different strains of wheat streak mosaic virus became infective and when transferred to healthy plants transmitted the different strains of the virus. Slykhuis (1955) states that mites became viruliferous within a period of 30 minutes on diseased plants. In this work to be certain that the mites fed and became viruliferous they were allowed to stay for 48 hours or more on the diseased plants before using them for transmission tests. The actual time that mites fed on the plants was not determined.

Five probable strains of wheat streak mosaic virus under code numbers

UK, SC, SD-1, MS and 41 were transmitted by A. tulipae (K.). One to ten mites were used for each test and it was found that a single mite was capable of transmitting the virus efficiently. The percentage of successful transmissions was essentially the same for the different strains indicating that each strain was transmitted with equal ease by the mite. The percent transmission varied from 84.21 to 92 for each strain of the virus (Tables 1 to 5 in Appendix). The average incubation period ranged from 7.04 to 8.68 days for the five isolates. This was the same incubation period for comparable temperatures as that reported by Sill and Fellows (1953) after manual inoculation. The symptoms produced after mite transmission of each strain were similar to those developing after manual inoculation of each strain. Based upon these results it would seem very likely that the five isolates of the virus tested were actually strains of the wheat streak mosaic virus.

In the greenhouse, very severe nonviruliferous mite infestations produced longitudinally rolled leaves, the tips of which became trapped in the rolled leaves and leafsheaths (Plate X) and produced chlorotic symptoms on otherwise healthy plants which under greenhouse conditions occasionally resulted in the death of the plants. This condition, however, was not transmissable manually and was never noticed in the field. This chlorosis of unknown significance was reported previously by Slykhuis (1955).

In a series of experiments with the MS strain using 142 hatched eggs from viruliferous mites, it was found that the nymphal forms hatching from those eggs when introduced to healthy plants did not carry the virus (Table 6). The data obtained would suggest that the eggs do not carry virus and only the nymphs and adults are capable of transmitting this strain of wheat streak mosaic virus. Since this strain is the most common one found in Kansas, it is probable that eggs do not carry any strain of the virus. This

also confirms the report of Slykhuis (1955) in which he used an undesignated strain and reported that the virus was not carried through the eggs.

EXPLANATION OF PLATE X

Pawnee wheat plants grown in 4-inch flower pots.

(a) Control, no mites introduced;

(b) Nonviruliferous mites introduced;

(c) Viruliferous mites placed on healthy plants.

Note the trapped leaves produced on the plants with mites.

PLATE X

(a) (b) (c)

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APPENDIX

Table 1. Transmission of strain 41 of wheat streak mosaic virus by Aceria tulipae Keifer.

Trial No.	No. of Plants Per Trial	No. of Mites Per Plant	No. of Plants Showing Symptoms	Incubation Period (days)
1	2	3	2	6
2	2	2 nymphs	2	7
3	2	7	1	7
4	2	5	2	7
5	2	1 nymph	2	7
6	2	4	none	none
7	2	6	none	none†
8	2	4	2	6
9	2	5	2	7
10	2	2	2	8
11	2	8	2	8
12	2	3	2	8
13	2	3	1	5
14	2	1	2	8
15	2	1 nymph	2	8
16	2	2	2	8
17	2	4	2	8
18	2	3	2	10
19	2	1	2	7
Total	38	65	32††	125
			Ave.	7.35

† Plants died after 2 days.
†† Total per cent transmission 84.21.

Table 2. Transmission of strain UK of wheat streak mosaic virus by Aceria tulipae Keifer.

Trial No.	No. of Plants Per Trial	No. of Mites Per Plant	No. of Plants Showing Symptoms	Incubation Period (days)
1	2	6	2	8
2	2	7	1	8
3	2	9	2	11
4	2	10	2	6
5	2	10	none	none
6	2	6	none	none
7	2	5	2	6
8	3	10	3	15
9	3	10	3	11
10	3	10	3	15
11	3	10	3	14
12	2	10	2	15
13	2	10	2	15
14	2	5	2	8
15	2	2	2	6
16	2	5	2	6
17	2	1	2	7
18	2	3 nymphs	2	6
19	2	5	2	6
20	2	2	2	6
21	2	10	2	6
22	2	5	2	5
23	2	1	2	6
24	2	2	2	5
Total	52	155	47‡‡	191
			Ave.	8.68

‡‡ Total per cent transmission 90.38.

Table 3. Transmission of strain SC of wheat streak mosaic virus by Aceria tulipae Keifer.

Trial No.	No. of Plants Per Trial	No. of Mites Per Plant	No. of Plants Showing Symptoms	Incubation Period (days)
1	2	10	2	6
2	2	10	2	5
3	2	10	2	7
4	2	10	2	13
5	2	10	none	none
6	2	10	2	5
7	2	10	2	9
8	2	10	2	7
9	2	10	2	10
10	2	2	2	5
11	2	10	2	6
12	2	10	2	11
13	2	5	2	6
14	2	10	2	12
15	2	7	2	10
16	2	5	2	7
17	2	5	1	6
18	2	3	2	5
19	2	3	2	5
20	2	5	2	6
21	2	1	2	5
22	2	5	2	5
23	2	3	1	6
24	2	2	2	5
25	2	3	2	5
Total	50	169	46‡‡	169
				Ave. 7.04

‡‡ Total per cent transmission 92.00.

Table 4. Transmission of strain SD-1 of wheat streak mosaic virus by Aceria tulipae Keifer.

Trial No.	No. of Plants Per Trial	No. of Mites Per Plant	No. of Plants Showing Symptoms	Incubation Period (days)
1	5	10	5	11
2	4	10	4	11
3	2	10	2	6
4	4	10	3	9
5	2	10	1	10
6	2	10	2	11
7	3	10	3	9
8	4	10	3	10
9	4	10	none	none[1]
10	4	10	4	8
11	3	10	3	7
12	2	1	2	7
13	2	3	2	8
14	2	5	2	9
15	2	2	2	8
Total	45	121	38[11]	124
				Ave. 8.13

1 The plants died after one week and no symptoms were observed in any of the plants in the trial.

11 Total per cent transmission 84.44.

Table 5. Transmission of strain MS of wheat streak mosaic virus by Aceria tulipae Keifer.

Trial No.	No. of Plants Per Trial	No. of Mites Per Plant	No. of Plants Showing Symptoms	Incubation Period (days)
1	2	2	2	4
2	2	10	2	7
3	2	3	2	11
4	2	5	2	7
5	2	1	2	11
6	2	10	2	6
7	2	10	none	none
8	2	10	2	10
9	2	10	2	14
10	2	10	2	6
11	2	10	2	7
12	3	10	2	4
13	2	10	2	9
14	2	10	2	5
15	2	10	2	5
16	2	2	2	6
17	2	2 nymphs	2	6
18	2	5	2	7
19	2	3	2	6
20	2	6	2	7
21	2	2	2	5
22	2	5	none	none
23	2	3	2	7
24	2	1	2	8
25	2	5	2	7
26	2	2 nymphs	2	7
Total	53	156	48[1]	172

[1] Total per cent transmission 90.56.

Table 6. Attempts to transmit wheat streak mosaic virus – MB strain– by eggs from viruliferous mites, Aceria tulipae Keifer.

Trial No.1/	No. of Eggs Placed	No. of Eggs Hatched	No. of Days for Eggs to Hatch2/	Symptoms Developed
1	6	5	2	none
2	5	3	1	"
3	5	2	3	"
4	9	8	2	"
5	12	10	3	"
6	10	8	1	"
7	10	9	1	"
8	10	7	2	"
9	10	none	none	none
10	10	8	3	"
11	10	7	1	"
12	10	8	3	"
13	10	9	1	"
14	10	6	4	"
15	10	6	2	"
16	10	9	1	"
17	10	6	3	"
18	10	9	2	"
19	10	8	1	"
20	10	6	2	"
21	10	8	3	"
Total	197	142	39††	

†† Total per cent hatched 72.08.

1/ One plant was used in each trial along with 2 sets of controls. In one set, no eggs were placed and in the other set, only eggs from non-viruliferous mites. No control plants developed symptoms.

2/ Eggs were examined daily for five days or until hatched before being discarded.

Table 7. Comparison of cotton and rubber sponges for plugs in inverted test tube cages.

Trial No.	No. of Mites Placed 1/	Number of Mites Survived After 3 Days	
		Cotton	Rubber sponge
1	5	none	5
2	10	"	9
3	2	"	2
4	2	"	2
5	7	"	6
6	6	"	6
7	3	"	2
8	1	1	1
9	1	none	none

1/ Individual mites were transferred by the use of human hair.

ACERIA TULIPAE (KEIFER) (ERIOPHYIDAE) IN RELATION TO THE TRANSMISSION OF VARIOUS STRAINS OF WHEAT STREAK MOSAIC VIRUS

by

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Aceria tulipae (Keifer) has been reported as the vector of wheat streak mosaic virus, a very serious disease in the Great Plains. In 1949 alone, the disease caused heavy losses amounting approximately to 30 million dollars. The mite is very small, about 270 microns in length, and cannot be seen by the naked eye. Its role as a vector was not recognized until Slykhuis (1953) demonstrated the relation of the mite in the spread of wheat streak mosaic virus. This report has been confirmed many times by workers in Kansas and Nebraska including the author and it is now certain that this mite is a major factor in the spread of this disease. This investigation was conducted primarily to discover whether the mite was an equally efficient vector of all known strains of the virus and secondarily to add wherever possible to our knowledge concerning the life cycle and habits of the mite.

The mite, A. tulipae (K.), was found to be an efficient vector for all strains of the virus tested. These strains were MS, UK, SC, SD-1 and 41. Briefly the techniques employed were as follows:

Nonviruliferous mites were placed on diseased wheat plants infected with various strains of the virus. These became viruliferous and when transferred again to healthy wheat plants transmitted the virus. The successful transmissions varied from 84.21 to 92 per cent in the different strains of wheat streak mosaic virus, and the incubation period ranged from 7.04 to 8.68 days. A. tulipae (K.), transmitted all the five isolates of the virus with equal efficiency and a single mite seemed to be as efficient in transmitting the virus as a larger number of mites. Symptoms after mite transmission were identical to those on plants inoculated manually. All three of these lines of evidence indicated that the five probable strains of the virus were actually strains of wheat streak mosaic virus.

A successful method of rearing mites under greenhouse conditions was

devised by using a series of different cages (described in the text) as early colonizing chambers. Rather than shifting individual mites, whole plants bearing mites were shifted from one cage to the next at the proper time. A highly humid environmental condition appeared to favor the rapid increase of mite populations.

Three successful methods of transferring mites are reported. A light (either incandescent or flashlight) was used successfully as one method of moving mites. The mites moved away when light was directed at them and tended to seek cover under the unexposed leaf areas. In all trials the mites appeared to be negatively phototropic. A medium coarse human hair was also found to be very convenient and was the most useful method of transferring the mites. A hollow end was used for picking up the eggs and a pointed tip for transferring or moving nymphs and adults. The third method was to insert portions of mite infested leaves into the youngest leaf axils of healthy plants. These were kept in place with a woman's hair clip. This method was not very practical as it was difficult to determine the exact time when the mites migrated to the new host plant. Also in many cases, the mites, for an unknown reason, would not migrate readily from the excised leaves to the new host and died in place.

On plants heavily infested with mites, trapped leaves and chlorotic symptoms were sometimes produced on virus-free plants in the greenhouse. This additional chlorotic factor previously reported by Slykhuis could not be transmitted manually. These chlorotic symptoms can be differentiated easily from wheat streak mosaic symptoms and were observed only in the greenhouse, never in the field.

The most common Kansas strain of the virus, MS, was not carried through

the eggs laid by viruliferous mites. One hundred and forty two eggs from viruliferous mites were placed on healthy wheat plants. The nymphs from these eggs did not transmit the virus and no symptoms of wheat streak mosaic ever developed on the test plants. This confirms the report of Slykhuis, who used an undesignated strain, that eggs do not carry this virus.

Mites on plants covered by water for long periods of time (2 days or more) died. It would appear therefore that prolonged flooding of infested plants might be utilized as a means of control. This technique, however, would probably never be suitable for field use but might be valuable at times in the greenhouse where only a few plants were involved.